REMARKS

I. Status of the Claims

Claims 45-61 are pending in this application.

Claim 54 has been amended to recite an "isolated" nucleic acid to distinguish the claimed nucleic acid molecule to any molecule that might exist naturally in a non-isolated form. Support for this amendment can be found throughout the specification, including, for example, at page 14, lines 12-21. The specification has also been amended at pages 23-24 to insert the SEQ ID NOs corresponding to the known sequences deposited at GenBank under the Accession Nos AE00159, AE000350, and J01713 and referenced at pages 23-24 of the specification.

This amendment does not add any new matter.

II. Objection to Specification

The Office objects to the amendment filed 2 November 2006 under 35 U.S.C. §132(a) alleging that it introduces new matter into the disclosure. Office Action at 2. According to the Office, the "additional matter which is not supported by the original disclosure is as follows: SEQ ID NOs 14-16." *Id.* The Office further asserts that if the sequences

> are the GenBank Accession Nos. referenced on pages 23 and 24 of the specification, in the context of the specification the Accession Nos. are used only to indicate where within the Accession Nos the specific primers in the PCR anneal, it is not even disclosed that the Accession Nos. were the actual PCR template (e.g. page 23, lines 19-26). Hence, the only support found for any sequences from these Accession Nos. is the inherent support that the PCR primers used in the specification anneal at specific regions in the Accession Nos. This does not provide support for the entire Accession Nos. which, at best, appear to be what is contained in the Sequence Listing.

Id.

Applicant respectfully traverses this objection. As noted by the Office, SEQ ID NOs 14, 15, and 16 correspond to the sequences deposited at GenBank under the Accession Nos AE00159, AE000350, and J01713, respectively. Pages 23-24 of the specification disclose that individual tRNA genes were isolated by PCR using primers that anneal at specific base pairs in the GenBank sequences AE00159, AE000350, and J01713. Thus, pages 23-24 teach one way to isolate *E. coli* tRNA genes using known nucleic acid sequences deposited and accessible from GenBank. Applicant's reference to these sequences by their Accession numbers provides an acceptable, short hand notation for referring to *known* nucleic acid sequences that complies with the written description requirement of 35 U.S.C. §112.

The Office's position that including the sequences of AE00159, AE000350, and J01713 in the sequence listing somehow introduces new matter into the specification because Applicant did not also set forth in the specification the known nucleotide sequences of AE00159, AE000350, and J01713 exalts form over substance and is directly at odds with Federal Circuit precedence on this issue. "Indeed, a requirement that patentees recite known DNA structures, if one existed, would serve no goal of the written description requirement. It would neither enforce the quid pro quo between the patentee and the public by forcing the disclosure of new information, nor would it be necessary to demonstrate to a person of ordinary skill in the art that the patentee was in possession of the claimed invention." *Falko-Gunter Falkner v. Inglis*, 448 F.3d 1357, 1368 (Fed. Cir. 2006). In *Falko-Gunter Falkner*, the Federal Circuit held that "where, as in this case, accessible literature sources clearly provided, as of the relevant date, genes and their nucleotide sequences . . ., satisfaction of the written description requirement does not require either the recitation or incorporation by reference (where permitted) of such genes and sequences." *Id.* Accordingly, SEQ ID NOs 14-16, corresponding to nucleotide sequences

referenced in the specification and accessible from literature sources (GenBank; *see also* Blattner *et al.*, Science 277(5331):1453-1474 (1997); Nakajima *et al.*, Cell 23(1):239-249 (1981)) as of the priority date, do not constitute new matter and do not require either the recitation or incorporation by reference of the specific nucleotide sequences. For these reasons, Applicant respectfully requests that the Office withdraw the objection to the amendment filed 2 November 2006 under 35 U.S.C. §132(a).

III. Rejections Under 35 U.S.C. §112, Second Paragraph

The Office rejects claims 50-52 and 59-61 under 35 U.S.C. §112, second paragraph, as allegedly "indefinite for failing to particularly point out and distinctly claim the subject matter the applicant regards as the invention." Office Action at 3. More specifically, the Office asserts:

> Claims 50-52 and 59-61 recite tRNA sequences comprising sequences found between specific base pairs of Genbank Accession Nos. The specification provides no guidance on which, if any, of the SEQ ID NOS in the Sequence Listing corresponds to the Genbank Accession Nos. recited in the claims.

Id. Applicant respectfully traverses this rejection.

Applicant has amended the specification at pages 23 and 24 to indicate that SEQ ID NO 14 corresponds to AE000159; SEQ ID NO:15 corresponds to AE000350; and J01713 corresponds to SEQ ID NO:16.

The Office also asserts that

> because the content of Genbank entries can (and do) change over time due to corrections, it cannot be determined if the sequences represented by the SEQ ID NOS (if they are the recited Genbank Nos.) are the actual DNA sequences as they existed at the time of filing of the instant application (applicants claim priority to 1999). Therefore, it cannot be determined what the actual tRNA sequences must be in order to anticipate the claims, preventing a satisfactory search of the claims.

Id.

Applicant acknowledges that GenBank entries can change over time due, for example, to corrections of the sequence. However, GenBank inserts warnings in its entries to alert readers when a sequence has changed as well as interactive links to find the altered sequence. For example, the GenBank entry for Accession No. AE000159 indicates that the sequence was replaced on 21 June 2004 by gi:48994873. *See* Attachment A. Thus, the 10,133 base pair sequence at Accession No. AE1000159, represented by SEQ ID NO:14, corresponds to the sequence as submitted and as it existed as of Applicant's priority date. The GenBank entry for Accession No. AE000350 indicates that the sequence replaced gi:178900 on 9 September 1997. *See* Attachment B. There is no indication that the sequence ever changed after 9 September 1997. Thus, the 10,401 base pair sequence at Accession No. AE1000350, represented by SEQ ID NO:15, corresponds to the sequence as it existed as of Applicant's priority date. Finally, the GenBank entry for Accession No. J01713 indicates that the 1100 base pair sequence has not changed since its submission. *See* Attachment C. Thus, the 1100 base pair sequence at Accession No. J01713, represented by SEQ ID NO:16, corresponds to the sequence as submitted and as it existed as of Applicant's priority date.

As evidenced by Attachments A-C, as of Applicant's priority date, the sequences represented by GenBank Accession Nos AE00159, AE000350, and J01713 are readily ascertainable and definite. As such, the metes and bounds of the claimed subject matter is clear, and Applicant respectfully requests that the Office withdraw this 35 U.S.C. §112, second paragraph rejection.

IV. Rejections Under 35 U.S.C. §112, First Paragraph

The Office rejects claims 50-52 and 59-61 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. Office Action at 3. Specifically, the Office asserts:

> The recitation of tRNA sequences comprising sequences of Genbank Accession Nos. in claims 50-52 and 59-61 is an attempt to incorporate essential, claimed subject matter by reference to prior art documents (i.e. the Genbank entries). A review of the disclosure as filed does not reveal these DNA sequences, thus there is no evidence applicants had possession of these specific DNA sequences. The incorporation of essential material in the specification by reference to an unpublished U.S. application, foreign application or patent, or to a publication is improper. See MPEP §608.01(p) (I). Applicant is required to amend the disclosure to include the material incorporated by reference, if the material is relied upon to overcome any objection, rejection, or other requirement imposed by the Office.

Id. at 4. Applicant respectfully traverses this rejection.

As noted above, there is no per se rule that written description requirement for nucleic acids can only be satisfied by a recitation of the actual nucleotide sequences in the specification. In fact, the Federal Circuit has held that requiring an Applicant to recite known DNA structures is at odds with the goals of the written description requirement:

> Indeed, a requirement that patentees recite known DNA structures, if one existed, would serve no goal of the written description requirement. It would neither enforce the quid pro quo between the patentee and the public by forcing the disclosure of new information, nor would it be necessary to demonstrate to a person of ordinary skill in the art that the patentee was in possession of the claimed invention. As we stated in Capon, "[t]he 'written description' requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution." *Id.* at 1358. Indeed, the forced recitation of known sequences in patent disclosures would only add unnecessary bulk to the specification.

Falko-Gunter Falkner, 448 F.3d at 1368.

The sequences represented by SEQ ID NOs 14-16 were known and accessible from GenBank and other references as of Applicant's priority date. Applicant refers to these known sequences in the specification using the shorthand Accession numbers: AE00159, AE000350, and J01713. The Federal Circuit has held that, as in this case, where "accessible literature sources clearly provided, as of the relevant date, genes and their nucleotide sequences . . ., satisfaction of the written description requirement does not require either the recitation or incorporation by reference (where permitted) of such genes and sequences." *Id.*; *see also*, *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 3323 F.3d 956, 966 (Fed. Cir. 2002) ("We therefore agree with Enzo that reference in the specification to deposits of nucleotide sequences describe those sequences sufficiently to the public for purposes of meeting the written description requirement."). Accordingly, Applicant is not required to recite the actual nucleotide sequences of these known nucleic acid molecules in the application; nor is Applicant required to incorporate them by reference. Applicant's reference in the application to the Accession numbers of these known and deposited sequences adequately satisfies the written description requirement. *See Falko-Gunter Falkner*, 448 F.3d at 1368. Thus, Applicant respectfully requests the Office to withdraw this written description rejection.

V. Rejections Under 35 U.S.C. §101

The Office rejects claims 54 and 58 under 35 U.S.C. §101 alleging that the subject matter is directed to non-statutory subject matter. Office Action at 10. The Office asserts that the "nucleic acid is not recited as purified or isolated, and thus reads on the *E. coli* genome, a product of nature." *Id.* Applicant has amended claim 54 to recite an "isolated" nucleic acid. Claims 55-61 depend directly or indirectly from claim 54. Accordingly, Applicant respectfully requests the Office to withdraw this rejection.

VI. Rejections Under 35 U.S.C. §103

A. *Del Tito* in Combination with *Nakamura, Zhang, Saier, Kawakami, Clouthier, and Sprinzl* Do Not Render Claims 45-48, 53-56, and 58 Obvious

The Office rejects claims 45-48, 53-56, and 58 under 35 U.S.C. §103(a) as allegedly obvious over Del Tito et al. ("*Del Tito*") in view of Nakamura et al. ("*Nakamura*"), Zhang et al. ("*Zhang*"), *Saier*, Kawakami et al. ("*Kawakami*"), Clouthier et al. ("*Clouthier*"), and Sprinzl et al. ("*Sprinzl*"). Office Action at 5-9. Applicant respectfully traverses this rejection.

To establish a *prima facie* case of obviousness, the prior art reference (or references when combined) must teach or suggest all the claim limitations. *See* M.P.E.P. § 2142. Applicant submits that the combined teachings of the cited references do not teach all of the elements of the rejected claims.

Claims 45 and 54[1] recite "sequences encoding *E. coli* argU, ileY, and leuW tRNAs." The Office acknowledges that *Del Tito* fails to teach the use of a vector comprising an array of the three tRNAs: argU, ileY, and leuW. Office Action at 6. However, the Office asserts:

> It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the vector construct taught by Del Tito et al for compensating for the presence of rarely used codons present in the gene encoding a protein of interest by interchanging and/or **adding different tRNA genes** corresponding to other rarely used codons in a given cell type, because Del Tito et al teach that it is within the skill of the art to carefully scrutinize the coding sequence of a protein, identify rarely used codons and compensate for the presence of such rarely codons by supplying in trans the RNA corresponding to the identified rarely used codons from a vector expressing different tRNA genes, and because the rarely used codons and corresponding genes were widely known in the art (i.e. the teachings of Nakamura et al, Zhang et al, Saier, Sprinzl et al, Kawakami et al and Clouthier et al). One would have been motivated to do so in order to meet the particular rare-codon requirements of a gene encoding a desired protein in combination

[1] The remaining claims under rejection depend directly or indirectly from independent claims 45 and 54.

> with a given cell type, and thus receive the expected benefit of increasing its expression in the given cell type, as taught by Del Tito et al.

Office Action at 7-8; emphasis added. Thus, the Office asserts that it would have been obvious to one of skill in the art to add different tRNA genes to the vectors of *Del Tito*. Applicant respectfully disagrees.

The *ileX* gene encodes a tRNA that recognizes the AUA codon, a codon that is rarely used in *E. coli*. To enhance the expression of heterologous proteins in *E. coli*, *Del Tito* teaches coexpressing the heterologous protein and the *ileX* gene. *Del Tito* inserted the *ileX* DNA into three pBR322-compatible vectors, one of which (pRI952) was a pACYC184 derivative containing a second tRNA gene, *argU*. *Del Tito* does not teach a vector or nucleic acid containing three tRNA genes. Moreover, *Del Tito* actually teaches away from adding a third tRNA gene to vectors for expressing heterologous proteins.

A prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 22 USPQ 303 (Fed. Cir. 1983). *See* M.P.E.P. § 2141.02. *Del Tito* observed less heterologous protein expression when using the pRI952 vector containing two tRNA genes (*ileX* and *argU*). *See De Tito*, page 7089, second column (Table 1). In addition, *Del Tito* notes that overexpression of tRNA genes is deleterious to the cell. *Id.* at 7090, second column, second paragraph. This is consistent with the specification, which states that "[o]verexpression of tRNA genes can be deleterious to the host cell, however. Rojiani et al., Proc. Nat. Acad. Sci. U.S.A. 87:1511-1515 (1990); Sharp et al., Nucleic Acids Res. 14:7737-7749 (1986)." Specification, page 3, lines 12-14. Thus, taken together and considered as a whole, *Del Tito* in combination with other art, like *Rojiani* and *Sharp*, teaches away from using three or more tRNAs in a host

cell to enhance heterologous gene expression. As indicated in *Del Tito*, expression of two tRNAs reduces heterologous protein expression as compared to vectors containing a single tRNA gene. Furthermore, *Del Tito* and other art references teach that overexpression of tRNA genes is deleterious to host cells. Thus, one of skill in the art would not have been motivated to increase the number and amount of tRNA gene expression because he would have expected such an increase to have a deleterious effect on the host cell.

Accordingly, the cited references fail to teach or suggest all of the elements of the claimed invention. For at least this reason, Applicant submits that the Office has not established a *prima facie* case of obviousness.

Furthermore, determining whether an invention is obvious from the prior art can require more than merely analyzing the differences between the prior art and the claimed invention. The Supreme Court has instructed that courts may also evaluate "secondary considerations" of obviousness, *i.e.*, other objective factors that may show that an invention was not obvious from the prior art, even if the differences between the prior art and the claimed invention seem slight. *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1, 17-18, 86 S. Ct. 684, 694, 15 L. Ed. 2d 545, 148 USPQ 459, 467 (1966) ("Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. As indicia of obviousness or nonobviousness, these inquiries may have relevancy." Indeed, "[i]t is the secondary considerations that are often the most probative and determinative of the ultimate conclusion of obviousness or nonobviousness." *Pro-Mold and Tool Co. v. Great Lakes Plastics, Inc.*, 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1630 (Fed. Cir. 1996) (reversing summary judgment of obviousness because district court failed to consider patentee's commercial success).

Here, the Office must consider the evidence of commercial success submitted in parent Application No. 09/492,590 (*see* Attachments D and E), evidence providing an objective indication of non-obviousness. Specifically, the Rule 132 Declarations report the significant sales figures of competent cells (BL21-CodonPlus™ RIL (Catalog # 230240); BL21-CodonPlus™ (DE3)-RIL (Catalog # 230245); and BL21-CodonPlus™ (DE3)-RIL-X (Catalog # 230265); the "RIL products") comprising a vector or nucleic acid having sequences encoding *E. coli* argU, ileY, and leuW tRNAs, as recited in the pending claims. As explained in the Rule 132 Declaration, the commercial success of the RIL products is due to the presence of three tRNA genes (i.e., argU, ileY, and leuW) encoding rarely used codons (*see* Attachment D at page 3, ¶2), thereby establishing a nexus between the commercial success and the claimed invention. *J.T. Eaton & Co. v. Atlantic Paste & Glue Co.*, 106 F.3d 1563, 1571, 41 USPQ2d 1641, 1647 (Fed. Cir. 1997) ("When a patentee can demonstrate commercial success, usually shown by significant sales in a relevant market, and that the successful product is the invention disclosed and claimed in the patent, it is presumed that the commercial success is due to the patented invention.")

Accordingly, for the reasons set forth above, Applicant requests that the Office reconsider and withdraw the 35 U.S.C. § 103 rejection of claims 45-48, 53-56, and 58 based on *Del Tito* in combination with *Nakamura*, *Zhang*, *Saier*, *Kawakami*, *Clouthier*, and *Sprinzl*.

B. *Del Tito* in Combination with *Nakamura, Zhang, Saier, Kawakami, Clouthier, Sprinzl*, and Further in View of *Skerra*, Do Not Render Claims 49 and 57 Obvious

The Office rejects claims 49 and 57 under 35 U.S.C. §103(a) as allegedly obvious over *Del Tito*, *Nakamura*, *Zhang*, *Saier*, *Kawakami*, *Clouthier*, and *Sprinzl* as applied to claims 45-48, 53-56, and 58 and further in view of U.S. Patent No. 5,849,576 to Skerra et al. ("*Skerra*"). Office Action at 9-10. Applicant respectfully traverses this rejection.

Applicant submits that the combined teachings of the cited references do not teach all of the elements of the rejected claim. As noted above, neither *Del Tito*, *Nakamura*, *Zhang*, *Saier*, *Kawakami*, *Clouthier*, nor *Sprinzl* teaches or suggests a nucleic acid or vector comprising sequences encoding *E. coli* argU, ileY, and leuW tRNAs. *Skerra* fails to remedy the deficiencies of *Del Tito*, *Nakamura*, *Zhang*, *Saier*, *Kawakami*, *Clouthier*, and *Sprinzl*. Accordingly, the cited references fail to teach or suggest all of the elements of the claimed invention. For at least this reason, Applicant requests that the Office reconsider and withdraw the 35 U.S.C. § 103 rejection of claims 49 and 57 based on *Del Tito*, *Nakamura*, *Zhang*, *Saier*, *Kawakami*, *Clouthier*, and *Sprinzl* in combination with *Skerra*.

VII. Conclusion

In view of the foregoing Amendment and Remarks, Applicant submits that this application is in condition for allowance. Applicant therefore requests entry of this Amendment, the Examiner's reconsideration and reexamination of the application, and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this paper and charge any additional required fees to Deposit Account No. 50-3740.

Respectfully submitted,
Carsten-Peter CARSTENS

Date: 27 December 2007

By: /Timothy B. Donaldson/
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Attachments:

GenBank Accession No. AE000159 printout from NCBI website (Attachment A)

GenBank Accession No. AE000350 printout from NCBI website (Attachment B)

GenBank Accession No. J01713 printout from NCBI website (Attachment C)

Copy of Rule 132 Declaration of Mary Buchanan from parent Application No. 09/492,590 dated 8 February 2001 (Attachment D)

Copy of Rule 132 Declaration of Mary Buchanan from parent Application No. 09/492,590 dated 8 November 2001 (Attachment E)

ATTACHMENT A

1: AE000159. Reports ...[gi:1786739] The record has been replaced by U00096

Comment Features Sequence

```
LOCUS       AE000159                10133 bp    DNA     linear   BCT 01-DEC-2000
DEFINITION  Escherichia coli K12 MG1655 section 49 of 400 of the complete
            genome.
ACCESSION   AE000159 U00096
VERSION     AE000159.1  GI:1786739
KEYWORDS    .
SOURCE      Escherichia coli K12
  ORGANISM  Escherichia coli K12
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Escherichia.
REFERENCE   1  (bases 1 to 10133)
  AUTHORS   Blattner,F.R., Plunkett,G. III, Bloch,C.A., Perna,N.T., Burland,V.,
            Riley,M., Collado-Vides,J., Glasner,J.D., Rode,C.K., Mayhew,G.F.,
            Gregor,J., Davis,N.W., Kirkpatrick,H.A., Goeden,M.A., Rose,D.J.,
            Mau,B. and Shao,Y.
  TITLE     The complete genome sequence of Escherichia coli K-12
  JOURNAL   Science 277 (5331), 1453-1474 (1997)
   PUBMED   9278503
REFERENCE   2  (bases 1 to 10133)
  AUTHORS   Blattner,F.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-JAN-1997) Guy Plunkett III, Laboratory of Genetics,
            University of Wisconsin, 445 Henry Mall, Madison, WI 53706, USA.
            Email: ecoli@genetics.wisc.edu Phone: 608-262-2534 Fax:
            608-263-7459
REFERENCE   3  (bases 1 to 10133)
  AUTHORS   Blattner,F.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-1997) Guy Plunkett III, Laboratory of Genetics,
            University of Wisconsin, 445 Henry Mall, Madison, WI 53706, USA.
            Email: ecoli@genetics.wisc.edu Phone: 608-262-2534 Fax:
            608-263-7459
REFERENCE   4  (bases 1 to 10133)
  AUTHORS   Plunkett,G. III.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-OCT-1998) Laboratory of Genetics, University of
            Wisconsin, 445 Henry Mall, Madison, WI 53706, USA
COMMENT     [WARNING] On Jun 21, 2004 this sequence was replaced by
            gi:48994873.
            This sequence was determined by the E. coli Genome Project at the
            University of Wisconsin-Madison (Frederick R. Blattner, director).
            Supported by NIH grants HG00301 and HG01428 (from the Human Genome
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            determined from E. coli K12 strain MG1655. Predicted open reading
            frames were determined using GeneMark software, kindly supplied by
            Mark Borodovsky, Georgia Institute of Technology, Atlanta, GA,
            30332 [e-mail: mark@amber.gatech.edu]. Open reading frames that
            have been correlated with genetic loci are being annotated with CG
            Site Nos., unique ID nos. for the genes in the E. coli Genetic
            Stock Center (CGSC) database at Yale University, kindly supplied by
            Mary Berlyn. A public version of the database is accessible
            (http://cgsc.biology.yale.edu). Annotation of the genome is an
            ongoing task whose goal is to make the genome sequence more useful
            by correlating it with other data.  Comments to the authors are
            appreciated. Updated information will be available at the E. coli
            Genome Project's World Wide Web site
            (http://www.genetics.wisc.edu). *** The E. coli K12 sequence and
            its annotations are periodically updated; this is version M54. No
            sequence changes. Annotation updates: updated gene identifications
```

and products; all new functional assignments courtesy of Monica Riley; added promoters, protein binding sites, and repeated sequences described in reference 1. The unique numeric identifiers beginning with a lowercase 'b' assigned to each gene (protein- or RNA-encoding) are now designated as gene synonyms instead of labels. This should allow them to be searched for in Entrez as gene names.

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                /function="RNA; tRNA"
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Aug 28 2007 16:53:42

ATTACHMENT B

1: AE000350. Reports ...[gi:2367147] The record has been replaced by U00096

Comment Features Sequence

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DEFINITION  Escherichia coli K12 MG1655 section 240 of 400 of the complete
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ACCESSION   AE000350 U00096
VERSION     AE000350.1  GI:2367147
KEYWORDS    .
SOURCE      Escherichia coli K12
  ORGANISM  Escherichia coli K12
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Escherichia.
REFERENCE   1  (bases 1 to 10401)
  AUTHORS   Blattner,F.R., Plunkett,G. III, Bloch,C.A., Perna,N.T., Burland,V.,
            Riley,M., Collado-Vides,J., Glasner,J.D., Rode,C.K., Mayhew,G.F.,
            Gregor,J., Davis,N.W., Kirkpatrick,H.A., Goeden,M.A., Rose,D.J.,
            Mau,B. and Shao,Y.
  TITLE     The complete genome sequence of Escherichia coli K-12
  JOURNAL   Science 277 (5331), 1453-1474 (1997)
  PUBMED    9278503
REFERENCE   2  (bases 1 to 10401)
  AUTHORS   Blattner,F.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-JAN-1997) Guy Plunkett III, Laboratory of Genetics,
            University of Wisconsin, 445 Henry Mall, Madison, WI 53706, USA.
            Email: ecoli@genetics.wisc.edu Phone: 608-262-2534 Fax:
            608-263-7459
REFERENCE   3  (bases 1 to 10401)
  AUTHORS   Blattner,F.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-1997) Guy Plunkett III, Laboratory of Genetics,
            University of Wisconsin, 445 Henry Mall, Madison, WI 53706, USA.
            Email: ecoli@genetics.wisc.edu Phone: 608-262-2534 Fax:
            608-263-7459
REFERENCE   4  (bases 1 to 10401)
  AUTHORS   Plunkett,G. III.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-OCT-1998) Laboratory of Genetics, University of
            Wisconsin, 445 Henry Mall, Madison, WI 53706, USA
COMMENT     On Sep 9, 1997 this sequence version replaced gi:1789000.
            This sequence was determined by the E. coli Genome Project at the
            University of Wisconsin-Madison (Frederick R. Blattner, director).
            Supported by NIH grants HG00301 and HG01428 (from the Human Genome
            Project and NCHGR). The entire sequence was independently
            determined from E. coli K12 strain MG1655. Predicted open reading
            frames were determined using GeneMark software, kindly supplied by
            Mark Borodovsky, Georgia Institute of Technology, Atlanta, GA,
            30332 [e-mail: mark@amber.gatech.edu]. Open reading frames that
            have been correlated with genetic loci are being annotated with CG
            Site Nos., unique ID nos. for the genes in the E. coli Genetic
            Stock Center (CGSC) database at Yale University, kindly supplied by
            Mary Berlyn. A public version of the database is accessible
            (http://cgsc.biology.yale.edu). Annotation of the genome is an
            ongoing task whose goal is to make the genome sequence more useful
            by correlating it with other data. Comments to the authors are
            appreciated. Updated information will be available at the E. coli
            Genome Project's World Wide Web site
            (http://www.genetics.wisc.edu). *** The E. coli K12 sequence and
            its annotations are periodically updated; this is version M54. No
            sequence changes. Annotation updates: updated gene identifications
            and products; all new functional assignments courtesy of Monica
```

```
          Riley; added promoters, protein binding sites, and repeated
          sequences described in reference 1. The unique numeric identifiers
          beginning with a lowercase 'b' assigned to each gene (protein- or
          RNA-encoding) are now designated as gene synonyms instead of
          labels. This should allow them to be searched for in Entrez as gene
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     3361 cccctccggt aatagtgaag tgagagagct tgttgtatcc ttcgatatca gtccctgcac
     3421 ccgtgttggc aactaaagca ctgcccgtct cctggttaac cccatgtgca ataccgccgg
     3481 tatagacaat cagcgagcct ccggcgctaa tattgctgcc aattgccgta ccatctttct
     3541 caacaacctg ccggctcccg gaggatataa ttgttgtgtc agctttcccg ccgcttttga
     3601 tattttgcgt tccgctgttg atattggtgc ctgtggctat gccataatta ttaatattct
     3661 gtgtgccacc attaattatg gtatttgtcg cgtttcctgc aacatccata accccgccat
     3721 tatctattcg ggtcgcatca gctttagcat tggttaaaac tgacattgtt cctttatctt
     3781 taataatcgt cttgtttgcc gaaccatatg cgtttatgtc taaatgacca ccgttttcca
     3841 gcaacacatt gtctgccacg tgattgtgga tggagaatgc accttcacta ttcgtaccgc
     3901 tcaccgtcgt accgttagtg ttagttttta aaattgcacc atcgtgctgg gtaacatttg
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     4141 atactctttg ctcaccgcca tttttaatgg ttgttgtttc tactgtgctc tgttcaacat
     4201 actgtcgacc accatttatg gttgtgttcg ttgccagact tccttgtact acgtcctgag
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6361 ttatcaaata ataccccctt gaactcattc acaaacgcat cttttttttc ttgaatatct
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6601 tttcttttag cgcattcaaa aaactgatcg gcattatttt tattcgataa tttttttagtt
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     9361 ttggtatcga tatcattcct gattatacct ttaactttat gggaattggg ggtagtggta
     9421 aaaatgacct ggattatccc tctgctgata tacgagcgaa gatcagtaaa gatatagaag
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    10381 ggagcgcatt tgagggtgta t
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Aug 28 2007 16:53:42

ATTACHMENT C

1: J01713, Reports E.coli tRNA opero...[gi:147952] Links

Comment Features Sequence

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LOCUS       ECOTGOP                  1100 bp    DNA     linear   BCT 18-APR-1994
DEFINITION  E.coli tRNA operon: supB-E tRNA gene cluster coding for 7 tRNAs.
ACCESSION   J01713
VERSION     J01713.1  GI:147952
KEYWORDS    glnU gene; glnV gene; leuW gene; metT gene; supB region; supB-E
            transfer RNA operon; supE region; transfer RNA; transfer RNA-Gln;
            transfer RNA-Leu; transfer RNA-Met.
SOURCE      Escherichia coli
  ORGANISM  Escherichia coli
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Escherichia.
REFERENCE   1  (bases 1 to 1100)
  AUTHORS   Nakajima,N., Ozeki,H. and Shimura,Y.
  TITLE     Organization and structure of an E. coli tRNA operon containing
            seven tRNA genes
  JOURNAL   Cell 23 (1), 239-249 (1981)
   PUBMED   6163550
REFERENCE   2  (bases 897 to 899)
  AUTHORS   Nakajima,N., Ozeki,H. and Shimura,Y.
  TITLE     Organization and structure of an E. coli tRNA operon containing
            seven tRNA genes
  JOURNAL   Cell 25, 581-591 (1981)
REFERENCE   3  (sites)
  AUTHORS   Nakajima,N., Ozeki,H. and Shimura,Y.
  TITLE     In vitro transcription of the supB-E tRNA operon of Escherichia
            coli. Characterization of transcription products
  JOURNAL   J. Biol. Chem. 257 (18), 11113-11120 (1982)
   PUBMED   6286682
REFERENCE   4  (bases 1 to 1100)
  AUTHORS   Ooi,T.
  JOURNAL   Unpublished (1983)
COMMENT     Original source text: Escherichia coli DNA.
            [2]  correction to [1] in corrigendum.
            [3]  sites; transcription initiation and termination. [4] sent on
            tape from Kyoto University. Entry kindly reviewed by H.Ozeki
            (29-AUG-1984). The second tRNA, previously called 'X-tRNA', has
            been tentatively identified as Leu-tRNA.
            All seven tRNAs are found on one primary transcript in vitro [1].
            The DNA (bp 895-920) could form a hairpin structure of 12 bp, which
            is consistent with rho-independent termination sites. In the RNA
            termination site 'ctt' (bp 920-922) the first 't' is preferred
            (70%), the 'c' is second (22%) and the second 't' accounts for 5%
            of the RNA terminations [3].
FEATURES             Location/Qualifiers
     source          1..1100
                     /organism="Escherichia coli"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:562"
     misc_RNA        151..920
                     /note="supB-E operon precursor RNA"
     tRNA            184..260
                     /product="tRNA-Met"
                     /note="codon recognized: AUG"
     tRNA            270..354
                     /product="tRNA-Leu"
                     /note="codon recognized: CUA"
     tRNA            378..452
                     /product="tRNA-Gln"
                     /note="codon recognized: CAA"
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                     /product="tRNA-Gln"
                     /note="codon recognized: CCU"
     tRNA            577..653
                     /product="tRNA-Met"
                     /note="codon recognized: CUA"
     tRNA            701..775
                     /product="tRNA-Gln"
                     /note="codon recognized: CAG"
     tRNA            813..887
                     /product="tRNA-Gln"
                     /note="codon recognized: AAU"
ORIGIN      113 bp upstream of HincII site; 15.5 min on K12 map.
        1 atggctgtag ggtgggaaaa aacacaaagt aaacaataat tgacgaatat agcgccacgc
       61 tgttcgcaac ctaaccaaac agtcactttc gagcaatctt ccttgaaaaa gaggttgacg
      121 ctgcaaggct ctatacgcat aatgcgcccc gcaacgccga taaggtatcg cgaaaaaaaa
      181 gatggctacg tagctcagtt ggttagagca catcactcat aatgatgggg tcacaggttc
      241 gaatcccgtc gtagccacca tctttttttg cgggagtggc gaaattggta gacgcaccag
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      421 attccctggt tcgaatccag gtacccagc catcttcttc gagtaagcgg ttcaccgccc
      481 ggttattggg gtatcgccaa gcggtaaggc acggttttt gataccggca ttccctgttt
      541 cgaatccagg tacccagcc atcgaagaaa caatctggct acgtagctca gttggttaga
      601 gcacatcact cataatgatg gggtcacagg ttcgaatccc gtcgtagcca ccaaattctg
      661 aatgtatcga atatgttcgg caaattcaaa accaatttgt tggggtatcg ccaagcggta
      721 aggcaccgga ttctgattcc ggcattccga ggttcgaatc ctcgtacccc agccaattta
      781 ttcaagacgc ttaccttgta agtgcaccca gttggggtat cgccaagcgg taaggcaccg
      841 gattctgatt ccggcattcc gaggttcgaa tcctcgtacc ccagccacat taaaaaagct
      901 cgcttcggcg agcttttgc ttttctgcgt tcattcaatg tcgaatgcga tgttgacacg
      961 tcttatcctt caatgtcgga tgcgacgctg ccgcgtctta tccgacctac ggttggcacg
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     1081 acgccagcac gctccgccgc
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ATTACHMENT D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#10

Application of:	Cartens, Carsten-Peter		
Serial No.:	09/492,590	Examiner:	G. Leffers Jr.
Filed:	January 27, 2000	Group:	1636
Entitled:	METHODS AND COMPOSITIONS FOR HIGH LEVEL EXPRESSION OF A HETEROLOGOUS PROTEIN WITH POOR CODON USAGE		

Attorney Docket No.: 25436/1340 [Formerly 4114/85530]

Assistant Commissioner for Patents
Washington, D.C. 20231

RULE 132 DECLARATION OF MARY BUCHANAN

I, Mary Buchanan, hereby declare that:

1. I am employed by Stratagene, the assignee of record of the above-referenced patent application. I hold the position of Director of Product Management at Stratagene.

2. Stratagene's CodonPlus™ products contain tRNA genes corresponding to rarely used codons. Stratagene presently sells six different BL21-CodonPlus™ bacterial strains for use in enhancing the expression of protein encoded by genes containing rarely used codons. Bacterial strains BL21-CodonPlus™ RIL (Catalog # 230240), BL21-CodonPlus™ (DE3)–RIL (Catalog # 230245), and BL21-CodonPlus (DE3)–RIL-X (Catalog # 230265), are embodiments of the invention claimed in claims 1-12 and 37. Each of the above competent cell products contains three tRNA genes corresponding to rarely used codons consisting of argU, ileY, and leuW. Bacterial strains BL21-CodonPlus™ RP (Catalog # 230250), BL21-CodonPlus™ (DE3)–RP (Catalog # 230255), and BL21-CodonPlus(DE3)–RP-X (Catalog # 230275), are embodiments of

the invention claimed in claims 39-41 and 44. Each of the above competent cell products contains two tRNA genes corresponding to rarely used codons consisting of argU and proL.

CodonPlus™ host cell products were the first commercially available compositions to successfully allow high level expression of genes encoding rarely used codons. Sales figures provided below indicate the commercial success of these embodiments of the claimed invention (Table I). The products became available in May 1999 in the United States and continue to sell today.

Table I

Year	Catalog #	Product	Number of tRNA genes	Date Introduced	Total Sales
1999	230240	BL21-CodonPlus™-RIL Competent Cells	3	5/24/99	$ 103,482
1999	230245	BL21-CodonPlus™(DE3)-RIL-Competent Cells	3	5/24/99	325,143
1999	230250	BL21-CodonPlus™-RP Competent Cells	2	8/27/99	18,764
1999	230255	BL21-CodonPlus™(DE3)-RP Competent Cells	2	8/27/99	46,052
				1999 Total	493,441
2000	230240	BL21-CodonPlus™-RIL Competent Cells	3	5/24/99	59,265
2000	230245	BL21-CodonPlus™(DE3)-RIL Competent Cells	3	5/24/99	226,181
2000	230250	BL21-CodonPlus™-RP Competent Cells	2	8/27/99	51,210
2000	230255	BL21-CodonPlus™(DE3)-RP Competent Cells	2	8/27/99	144,118
2000	230265	BL21-CodonPlus™(DE3)-RIL-X Competent Cells	3	1/14/00	5,317
2000	230275	BL21-CodonPlus™(DE3)-RP-X Competent Cells	2	1/14/00	3,429
				2000 Total	489,520
2001	230240	BL21-CodonPlus™-RIL Competent Cells	3	5/24/99	5,174
2001	230245	BL21-CodonPlus™(DE3)-RIL Competent Cells	3	5/24/99	17,230
2001	230250	BL21-CodonPlus™-RP Competent Cells	2	8/27/99	3,377
2001	230255	BL21-CodonPlus™(DE3)-RP Competent Cells	2	8/27/99	15,610
2001	230265	BL21-CodonPlus™(DE3)-RIL-X Competent Cells	3	1/14/00	162
2001	230275	BL21-CodonPlus™(DE3)-RP-X Competent Cells	2	1/14/00	162
				2001 Total	41,715
				TOTAL:	$ 1,024,676

The primary reason one would use the CodonPlus™ series host cells is to increase the expression of genes containing low frequency codons. The CodonPlus™ series host cells are sold at $195 per ml, which is 44.4% higher than the same competent cells without the array of tRNA genes (BL21, Catalog # 200133 and BL21 (DE3), Catalog # 200131). Essentially all of the customers who purchased the CodonPlus™ cells used them for the purpose of increasing the expression of heterologous genes encoding rarely used codons because there is no reason to use the more expensive CodonPlus host strains for routine expression of genes without low

frequency codon problems. Therefore, the commercial success of the CodonPlus™ series host cells is due to the presence of an array of two or three tRNA genes encoding rarely used codons.

3. The market for bacterial host cells is shared by a number of major companies, including Invitrogen, Promega, Novagen, and Life Technologies. The consumer is thus free to choose a product on the basis of objective principles. The commercial success Stratagene enjoyed in selling CodonPlus™ host cells is not the result of heavy promotion or advertising, nor the result of consumption by purchasers normally tied to Stratagene. Stratagene spent no more on promotion and advertising of the CodonPlus™ host cells than it did on any of the other new competent cell products it sells. For example, XL10-Gold® Ultracompetent cells (Catalog Nos. 200314, 200315 and 200317) were released in May 1997. Sales in 1997 were $188,474 worldwide, $592,193 in 1998. Launch costs were similar for both products.

4. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

February 8, 2001
Date

Mary Buchanan
Director of Product Management
Stratagene
11011 North Torrey Pines Road
La Jolla, CA 92037

ATTACHMENT E

Attorney Docket No.: 25436/1340 [Formerly 4114/85530]

Express Mail Label NO.: EL326923155US
Date of Deposit: November 9, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Carstens, Carsten-Peter		
Serial No.:	09/492,590	Examiner:	G. Leffers Jr.
Filed:	January 27, 2000	Group:	1636
Entitled:	METHODS AND COMPOSITIONS FOR HIGH LEVEL EXPRESSION OF A HETEROLOGOUS PROTEIN WITH POOR CODON USAGE		

Assistant Commissioner for Patents
Washington, D.C. 20231

SUPPLEMENTAL DECLARATION OF MARY BUCHANAN

UNDER RULE 1.132

I, Mary Buchanan, hereby declare, supplemental to the Rule 1.132 Declaration filed on February 9, 2001, that:

1. I am employed by Stratagene, the assignee of record of the above-referenced patent application. I hold the position of Director of Product Management at Stratagene.

2. I understand that the Examiner has raised a question as to the relative level of sales of related products available in the marketplace upon which to base a comparison of the commercial success of the claimed host cell strains embodied in Stratagene's CodonPlus™ product line. The sales figures presented in my prior declaration encompassed Stratagene's sales over approximately 20 months from the first offer of sale in May of 1999 (BL21-CodonPlus™-RIL Competent Cells) to shortly before February 9, 2001. To my knowledge, prior to 2001, Stratagene held 100% of the market share for codon-enhanced host cells because there were no

other commercially available host cell strains containing recombinant genes for tRNAs specific for low-frequency codons (Novagen entered the market in early 200[illegible] with their Rosetta™ strains). There would be no reason to buy or use the more expensive CodonPlus™ strains for the expression of genes without codon bias problems, so comparison of sales figures from other expression systems that are not codon optimized would not provide a meaningful background against which to determine commercial success.

3. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

11/8/01
Date

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